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Review

Characterizing the human equivalent dose of herbal medicines in animal toxicity studies ☆

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ABSTRACT

Ethnopharmacological relevance: Herbal medicines have been generally believed to be safe. With the increasing use of herbal medicine worldwide, however, the safety of traditional herbal drugs frequently becomes a medical issue.
Aim of the study: This study was aimed to characterize the safe dose of herbal medicines through the systematic review for "human equivalent dose (HED)" from animal-based toxicity studies.
Methods and materials: A literature search for animal-based toxicity studies of herbal medicines in eight databases, including PubMed and Embase, was performed without language restriction. From the "no observed adverse effect level (NOAEL)" of each animal study, HED values were then calculated according to the composition (single or multiple herbs) and indication of the medicines.
Results: Among 729 relevant articles identified in the initial screening, 112 (233 studies comprising 105 single-herb and 128 multiple-herb studies) that met our inclusion criteria were finally reviewed. The total average HED value (from mouse, rat, rabbit and dog) was 278.1 ± 358.0 mg/kg, and the values for single- and multiple-herb studies were 322.7 ± 488.4 mg/kg and 241.5 ± 189.2 mg/kg, respectively. When the studies were analyzed according to herbal drug indication, drugs used for revitalization had the highest HED value (433.0 ± 265.2 mg/kg), while those for infectious diseases had the lowest (110.6 ± 118.6 mg/kg).
Conclusions: Our results provide important information regarding the safe dose of herbal medicines; thus, these data offer researchers and practitioners information critical for drug development or clinical application.

Contents

☆ The English in this document has been checked by at least two professional editors, both native speakers of English. For a certificate, please see: http://www.textcheck.com/certificate/Xn5WH3

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1. Introduction

The World Health Organization (WHO) has reported that approximately three-quarters of the world's population use herbal medicines for their healthcare (Gilani and Rahman, 2005). The market for herbal medicines is continuously expanding worldwide, and comprised US $83 billion globally in 2012 (WHO, 2013). The safety and efficacy of herbal products have been guaranteed usually based on their long history of clinical application (Neergheen-Bhujun, 2013). However, increasing concern exists regarding the lack of scientific evidence for the safety and efficacy of herbal medicines. Recently, several studies have warned of the possibility of herbal-drug-associated toxicity (Raynor et al., 2011; Posadzki et al., 2013).

Along with the attention paid to the safety of herbal medicines, the number of toxicity studies of herbal medicines is increasing (Kim et al., 2013). However, the number of toxicity studies is lacking, and several have presented controversial data. In Korea, for example, one study indicated herbal medicines as the major cause of drug-induced liver disease (Suk et al., 2012); however, another prospective study reported clinical data that suggested the safety of herbal medicines (Jeong et al., 2012). Information regarding the incidence and mechanism of herbal toxicity remains vague (Bent, 2008). Herbal drug-associated toxicity could have multiple causes, including direct toxic effects of the herb, environmental factors, and the genetic background of subjects (Haller et al., 2002).

To clarify the toxic effects of herbal medicines, the epidemiology of herbal-drug toxicity and its risk factors should be scientifically investigated. Nonetheless, toxicity studies of herbal medicines have been often neglected due to the perception that herbal agents are safe because they are natural products and have a long history of use (Ye and He, 2010). The pharmacological and toxicological processes are usually based on animal studies and clinical evaluation (Afolabi et al., 2012). Animal toxicity studies are important for predicting side effects and deciding the safe dose of drugs before clinical studies (Ali et al., 2012); thus, animal toxicity studies are the "gold standard" for toxicity assessment (de Broe and Porter, 2008).

In animal toxicity studies, accessing the "no observed adverse effect level (NOAEL)" is a fundamental process (Dorato and Engelhardt, 2005). The NOAEL value indicates the highest dose level not producing a significant increase in adverse effects in the experimental animal. From the NOAEL value, the human equivalent dose (HED) and maximum recommending starting dose (MRSD) can be calculated; these provide core information regarding the safety range and toxic potential of certain clinical doses of drugs, including herbal products (FDA Guidance, 2005).

Many animal toxicity studies for herbal plants or herbal formula were conducted to date, however no investigation showing the overview of those data was done yet. This study aimed to characterize the NOAEL and HED values of herbal medicines through a systematic survey of toxicity studies conducted to date worldwide.

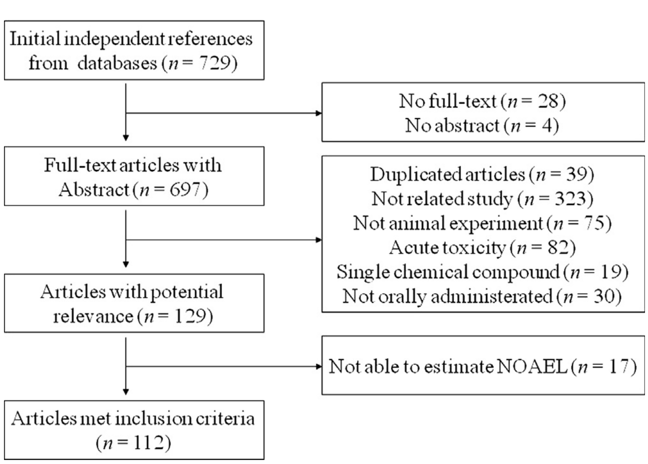

Fig. 1. Schematic of the data selection process.

2. Methods and materials

2.1. Data sources and key words

We searched the following eight databases: PubMed (http://www.ncbi.nlm.nih.gov/pubmed/), Embase (www.embase.com/), KISS (http://kiss.kstudy.com/), RISS (http://www.riss.kr/), KISTI (http://www.kisti.re.kr/), National Assembly Library (http://www.nanet.go.kr/), OASIS (http://oasis.kiom.re.kr/), and KMbase (http://kmbase.medric.or.kr/) from their inception to January 31, 2014. Search terms comprised combinations of the following keywords: "herbal," "plant," "safety," "toxicity," "hepatotoxicity," and "NOAEL." No limitation of article type, publication status, or language (if an abstract is written in English) was imposed. Korean terms adopted from the above were retrieved from Korean databases.

2.2. Eligibility criteria

Articles were screened using the following inclusion criteria: (1) animal study, (2) toxicity study, (3) herbal resource, (4) oral administration of sample, and (5) articles that can estimate NOAEL. The exclusion criteria were as follows: (1) article without the full text or abstract, (2) article concerning other aspects such as an efficacy or a carcinogenicity study, (3) human study or review article, (4) acute toxicity or in vitro study, (5) single chemical compound, and (6) other route except oral administration. In addition, studies that cannot estimate NOAEL were excluded.

The title and abstract of each searched article was initially read by two authors simultaneously. Four authors decided the articles that met the inclusion criteria.

2.3. Data extraction and summarization

The authors thoroughly read the selected articles and extracted data regarding the type of herbal medicine (single or multiple herbs), species and gender of animal, treatment period, name of herb and clinical indication, and dose of sample, to calculate NOAEL value. Toxicity tests were classified as sub-acute (two to five weeks), sub-chronic (over 5–14 weeks) and chronic (over six months) according to the regulatory guidelines of various international organizations (Prieto et al., 2006). We counted male and female studies individually and each type of study when more than two were reported by a single article.

If absent, the clinical use information was obtained from both the selected articles and other sources (public web-based databases and books). Herbal medicines were classified based on the International Classification of Diseases-10 (ICD-10, WHO, 2010), with slight modifications, as follows: digestive system, endocrine, nutritional and metabolic diseases, genitourinary system, mental and behavioral disorders, respiratory system, infectious diseases, neoplasms, circulatory system, anti-inflammation, analgesic, revitalization, and others. Some herbal medicines were classified into more than one category (but less than four).

2.4. Determination of NOAEL, HED, and MRSD

Regarding NOAEL determination, the NOAEL value was obtained from the indicated article; however, it was calculated according to Food and Drug Administration (FDA) instructions if no NOAEL value was provided (FDA Guidance, 2005). HED and MRSD values were obtained based on the NOAEL value according to the following formulae: HED = animal NOAEL/conversion factor; MRSD = HED/safety factor 10.

2.5. Statistical analysis

We compared the HED values of single- and multiple-herb studies, and between males and females, using independent *t*-tests. In addition, HED values were compared among 11 clinical indications of the drugs using analysis of variance. A P-value less than 0.05 was deemed to indicate statistical significance. All statistical analyses were performed using SPSS for Windows (SPSS® 18.0 KO; SPSS, Inc., Chicago, IL, USA).

3. Results

3.1. Characteristics of data

Among 729 relevant articles from the initial screening, 112 met the inclusion criteria (Fig. 1). These comprised 233 studies, of which 105 concerned a single herb and 128 multiple herbs. Total number of herbal drugs included was 110; 48 of single herb (Supplementary Table 1) and 62 of multiple herbs (Supplementary Table 2) respectively.

Sub-chronic studies were most frequent (121 studies, 51.9%), followed by sub-acute studies (96 studies, 41.2%) and chronic studies (16 studies, 6.9%, Table 1). The following four species of animal were used: 183 rats (114 for single herb and 69 for multiple herbs, total 78.5%), 37 mice (29 for single herb and 8 for multiple herbs, total 15.9%), 12 dogs (6 for single herb and 6 for multiple herbs, total 5.2%), and 1 rabbit (1 for single herb, 0.4%). The gender distribution was 128 male studies and 105 female studies (54.9% vs. 45.1%, Table 1).

3.2. Analysis of NOAEL and HED values

We calculated the NOAEL values for male and female studies, and single-herb and multiple-herb studies separately, to determine the

Table 1
Characteristics of the studies selected from articles.

Drugs	Animals		Sub-acute[a]	Sub-chronic	Chronic	Sum
Single herb	Rodent	Mouse (Male/Female)	16 (9/7)	9 (5/4)	4 (3/1)	29 (17/12)
		Rat (Male/Female)	34 (21/13)	33 (15/18)	2 (1/1)	69 (37/32)
	Non-rodent	Rabbit (Male/Female)	1 (1/0)	0	0	1 (1/0)
		Dog (Male/Female)	4 (2/2)	2 (1/1)	0	6 (3/3)
Total (Male/Female)			55 (33/22)	44 (21/23)	6 (4/2)	105 (58/47)
Multiple herbs	Rodent	Mouse (Male/Female)	4 (3/1)	4 (2/2)	0	8 (5/3)
		Rat (Male/Female)	35 (21/14)	71 (37/34)	8 (4/4)	114 (62/52)
	Non-rodent	Dog (Male/Female)	2 (1/1)	2 (1/1)	2 (1/1)	6 (3/3)
Total (Male/Female)			41 (25/16)	77 (40/37)	10 (5/5)	128 (70/58)
Total no. of studies[b] (Male/Female)			96 (58/38)	121 (61/60)	16 (9/7)	233 (128/105)

[a] The type of toxicity study was defined as follows: 2 to 5 weeks, sub-acute; more than 5 to 14 weeks, sub-chronic; more than 6 months, chronic.
[b] Studies were counted individually according to the type of study, animal, gender, and herbal medicine.

Table 2
Average NOAEL and HED values in males.

Type	**Animals**			**Sub-acute** [a]	**Sub-chronic**	**Chronic**	**Average**	**MRSD**
Single Herb	Rodent	Mouse	NOAEL	975.6 ± 1084.5 (9) [b]	1380.0 ± 2109.7 (5)	1833.3 ± 2742.4 (3)	1245.9 ± 1659.0 (17)	10.3 ± 13.5
			HED	79.3 ± 88.2	112.2 ± 171.5	149.1 ± 223.0	102.6 ± 134.9	
		Rat	NOAEL	1583.5 ± 1851.9 (21)	1856.5 ± 2681.4 (15)	5000.0 (1)	1786.5 ± 2239.3 (37)	28.7 ± 36.1
			HED	255.4 ± 298.7	299.4 ± 432.5	806.5	287.2 ± 361.2	
	Non-rodent	Rabbit	NOAEL	780.0 (1)	-	-	780.0 (1)	25.2
			HED	251.6			251.6	
		Dog	NOAEL	3500.0 ± 707.1 (2)	3000.0 (1)	-	3333.3 ± 577.3 (3)	185.2 ± 32.1
			HED	1944.4 ± 392.8	1666.7		1851.9 ± 320.8	
Average HED				309.6 ± 496.5 (33)	320.0 ± 488.5 (21)	313.4 ± 375.7 (4)	313.6 ± 479.1 (58)	31.4 ± 47.9
Multiple Herbs	Rodent	Mouse	NOAEL	4500.0 ± 4821.8 (3)	3400.0 ± 3677.0 (2)	-	4060.0 ± 3920.2 (5)	33.0 ± 31.9
			HED	365.9 ± 392.0	275.4 ± 298.9		330.1 ± 318.7	
		Rat	NOAEL	1338.3 ± 1376.3 (21)	1630.2 ± 976.5 (37)	1887.5 ± 2231.0 (4)	1547.9 ± 1206.4 (62)	25.0 ± 19.5
			HED	215.9 ± 222.0	262.9 ± 157.5	304.4 ± 359.8	249.7 ± 194.6	
	Non-rodent	Dog	NOAEL	400.0 (1)	400.0 (1)	100.0 (1)	300.0 ± 173.2 (3)	16.7 ± 9.6
			HED	222.2	222.2	55.6	166.7 ± 96.2	
Average HED				234.1 ± 274.5 (25)	262.6 ± 168.9 (40)	254.7 ± 330.9 (5)	251.9 ± 201.0 (70)	25.2 ± 20.1
Total HED value				277.1 ± 404.4 (58)	282.3 ± 311.0 (61)	280.8 ± 329.6 (9)	279.8 ± 354.9 (128)	28.0 ± 35.5

NOAEL, no observed adverse effect level; HED, human equivalent dose; MRSD, maximum recommended starting dose.
[a] The type of toxicity study was defined as follows: 2 to 5 weeks, sub-acute; more than 5 to 14 weeks, sub-chronic; more than 6 months, chronic.
[b] The mean ± standard deviation is expressed as mg/kg/day, and the number of studies is shown in parentheses.

Table 3
Average NOAEL and HED values in females.

Animals				Sub-acute [a]	Sub-chronic	Chronic	Average	MRSD
Single Herb	Rodent	Mouse	NOAEL	770.0 ± 881.8 (7) [b]	1355.0 ± 2431.3 (4)	5000.0 (1)	1317.5 ± 1860.2	10.7 ± 15.1
			HED	62.6 ± 71.7	110.2 ± 197.7	406.5	107.1 ± 151.2	
		Rat	NOAEL	1190.8 ± 737.0 (13)	1911.2 ± 2628.9 (18)	5000.0 (1)	1715.0 ± 2118.0 (32)	27.7 ± 34.2
			HED	192.1 ± 118.9	308.3 ± 424.0	806.5	276.6 ± 341.6	
	Non-rodent	Dog	NOAEL	3500.0 ± 707.1 (2)	3000.0 (1)	-	3333.3 ± 577.4 (3)	185.2 ± 32.1
			HED	1944.4 ± 392.8	1666.7		1851.9 ± 320.8	
Average HED				310.18 ± 548.0 (22)	255.3 ± 484.4 (23)	606.5 ± 282.8 (2)	333.9 ± 504.5 (47)	33.4 ± 50.5
Multiple Herbs	Rodent	Mouse	NOAEL	2500.0 (1)	3400.0 ± 3677.0 (2)	-	3100.0 ± 2651.4 (3)	25.2 ± 21.6
			HED	203.3	276.4 ± 298.9		252.0 ± 215.6	
		Rat	NOAEL	1090.4 ± 1373.4 (14)	1522.3 ± 808.2 (34)	1887.5 ± 2231.0 (4)	1434.1 ± 1104.7 (52)	23.1 ± 17.8
			HED	175.9 ± 221.5	245.5 ± 130.4	304.4 ± 359.8	231.3 ± 178.2	
	Non-rodent	Dog	NOAEL	400.0 (1)	400.0 (1)	100.0 (1)	300.0 ± 173.2 (3)	16.7 ± 9.6
			HED	222.2	222.2	55.6	166.7 ± 96.2	
Average HED				180.5 ± 200.2 (16)	246.6 ± 134.6 (37)	254.7 ± 330.9 (5)	229.0 ± 174.9 (58)	22.9 ± 17.5
Total HED value				255.6 ± 436.9 (38)	279.6 ± 316.8 (60)	355.2 ± 340.3 (7)	276.0 ± 363.5 (105)	27.6 ± 36.4

NOAEL, no observed adverse effect level; HED, human equivalent dose; MRSD, maximum recommended starting dose.

[a] The type of toxicity study was defined, 2 to 5 weeks as sub-acute, over 5 to 14 weeks as sub-chronic, and over 6 months as chronic.

[b] The mean ± standard deviation is expressed as mg/kg/day, and the number of studies is shown in parentheses.

HED values. In male studies, the average NOAEL values ranged from 300.0 ± 173.2 mg/kg to 4,060.0 ± 3,920.2 mg/kg in the four animal species. The average HED values for sub-acute, sub-chronic, and chronic studies were 277.1 ± 404.4 mg/kg, 282.3 ± 311.0 mg/kg, and 280.8 ± 329.6 mg/kg, respectively. The total average HED value was 279.8 ± 354.9 mg/kg (313.6 ± 479.1 mg/kg for single-herb studies and 251.9 ± 201.0 mg/kg for multiple-herb studies). There was no significant difference between single- and multiple-herb studies ($P > 0.05$, Table 2).

Regarding female studies, the average NOAEL values ranged from 300.0 ± 173.2 mg/kg to 3,333.3 ± 577.4 mg/kg. The average HED values for sub-acute, sub-chronic, and chronic studies were 255.6 ± 436.9 mg/kg, 279.6 ± 316.8 mg/kg, and 355.2 ± 340.3 mg/kg, respectively. The total average HED was 276.0 ± 363.5 mg/kg (333.9 ± 504.5 mg/kg for a single herb and 229.0 ± 174.9 mg/kg for multiple herbs). There was no significant difference between single- and multiple-herb studies ($P > 0.05$, Table 3). No significant difference was observed between male and female studies ($P > 0.05$).

3.3. *HED value according to clinical indication of drugs*

When the studies were classified according to clinical indication, statistical analysis showed significant differences in HED values among the drug classifications for 11 indications ($P < 0.01$). Herbal drugs for the digestive system (54 for single- and multiple-herb medications) were the most frequent, followed by herbal drugs for anti-inflammation (37), the genitourinary system (36), analgesic purposes (33), and the endocrine system, nutritional and metabolic diseases (32), respectively. Herbal drugs for revitalization had the highest HED value (433.0 ± 265.2 mg/kg), followed by herbal drugs for mental and behavioral disorders (391.6 ± 570.8 mg/kg), analgesic purposes (343.3 ± 226.6 mg/kg), and the respiratory system (238.7 ± 222.5 mg/kg), respectively. Herbal drugs for infectious diseases showed the lowest HED value (110.6 ± 118.6 mg/kg, Table 4).

4. Discussion

Herbal medicines or medicinal plants are the major constituents of various traditional medicines in Traditional Chinese Medicine (TCM), Ayurveda, and homeopathy (Bandaranayake, 2006). With the increasing use of herbal remedies worldwide, scientific evaluation of the benefits and risks of commonly used herbs is required (Ernst, 2002). Particularly, warnings concerning possible herbal-drug-associated toxicity drove an increase in research on herbal drug toxicity (Bunchorntavakul and Reddy, 2013).

In the current systematic review, we searched animal studies of herbal drug toxicity, and calculated the average NOAEL and HED values to estimate the general safety level of herbal medicines. One hundred and twelve toxicity articles met the inclusion criteria, and 233 studies were included in the final analysis. Additionally, the studies were stratified according to gender, animal species, and study period. The number of animal-based toxicity evaluations has increased rapidly since 2000, comprising 84.8% of articles on herbal medicine toxicity (data not shown). Among the 233 studies, single-herb studies accounted for 105 (45.1%), and multiple-herb studies for 128 (54.9%); the majority of studies used the rat (78.5%). The majority of studies were conducted in China, Korea, Japan, India, and the United States (data not shown). The latter finding might be due to the more popular use of herbal medicines in Eastern countries; additionally, Korean and international databases were included.

Herbal drug toxicity could be mediated by multiple mechanisms, including direct toxic effects of the herb, allergic reactions, effects of contaminants, and interactions with drugs or other herbs (Bent and Ko, 2004). Among those factors, information on the risk of direct toxicity is essential to obtain safety information for any drug (Woo et al., 2012). Because toxicity is generally expected to follow a dose–response pattern, determination of the HED value from the NOAEL value in animal studies is the basis of knowledge of the safety of drugs at clinical doses (Dorato and Engelhardt, 2005).

The average HED value of the 233 studies was 278.1 ± 358.0 mg/kg. Traditional herbal formulas consisting of multiple herbs generally employ a larger clinical dose than single-herb drugs; they are believed to be safer than single-herb drugs (Kiyohara et al., 2004; Efferth and Kaina, 2011). Therefore the larger HED value in multiple-herb than single-herb studies was expected. The HED value for multiple-herb medicines (241.5 ± 189.2 mg/kg), however, was lower than that for single-herb medicines (322.7 ± 488.4 mg/kg) in our results, even this difference was not statistically significant ($P > 0.05$). In our study, NOAEL values had a wide range, from 5 mg/kg for Trikatu, an Ayurvedic herbal product (Chanda et al., 2008), to 10,308 mg/kg for *Zea mays* L. (corn silk), a Chinese herb (Wang et al., 2011). Trikatu is an Ayurvedic formula consisted of three herbs (*Piper nigrum, Piper longum, Zingiber officinalis*) for digestive and respiratory disorder (Johri and Zutshi,1992), while *Zea mays* L is a single herb for various disorders in urinary system (Wang et al., 2012). Our data for HED value were in accordance with the known facts; for example *Aristolochia contorta* Bunge (34.4 mg/kg of HED) and *Piper methysticum* G. Forst

Table 4
Average HED value by drug indication.

Indication of drugs Average HED (no. of studies) [a]	Names of Single and Multiple Herbs
Digestive system 228.4 ± 278.2 (54) [b]	Single: *Akebia quinata* (Houtt.) Decne., *Akebia trifoliate* (Thunb.) koidz., *Aristolochia manshuriensis* Kom., *Aster squamatus* (Spreng.) Hieron., *Bambusa tuldoides* Munro, *Chelidonium majus* L., *Hippophae rhamnoides* L., *Ludwigia octovalvis* (Jacq.) P.H.Raven, *Nelumbo nucifera* Gaertn., *Plumeria rubra* L., *Rheum palmatum* L., *Senecio scandens* Buch.-Ham. ex D.Don, *Tephrosia purpurea* (L.) Pers. Multiple: Amukkarac curanam, CGX, CIRUELAX®, DA-9701, DAS-77, HZJW, Keishi-ka-shakuyaku-to, Longdan Xieganwan, Ojeok-san, PAI-N00, Rikkunshi-to, Shigyaku-san, Soshiho-tang, Tonica, Trikatu
Anti-inflammation 146.1 ± 127.9 (37)	Single: *Asarum heterotropoides* F.Schmidt, *Bambusa tuldoides* Munro, *Bryophyllum pinnatum* (Lam.) Oken, *Commiphora myrrha* (Nees) Engl., *Cyperus rotundus* L., *Dioscorea villosa* L., *Moringa oleifera* Lam., *Nelumbo nucifera* Gaertn., *Plumeria rubra* L., *Pteris multifida* Poir., *Rhododendron arboreum* Sm., *Stachys lavandulifolia* Vahl Multiple: Habb-e-Asgand, Homnawakod, Huo Luo Xiao Ling Dan, Operation Sweep, Sairei-to, SKI306X, Soshiho-tang, Tonica
Genitourinary system 234.0 ± ± 358.0 (36)	Single: *Cassytha filiformis* L., *Dioscorea villosa* L., *Leonurus japonicus* Houtt., *Marantodes pumilum* (Blume) Kuntze, *Nigella damascena* L., *Serenoa repens* (W.Bartram) Small, *Stachys lavandulifolia* Vahl, *Zea mays* L. Multiple: Amukkarac curanam, DAS-77, Etana, Gosha jinki-gan, Hachimi-jio-gan, Keishi-bukuryo-gan, Sairei-to, Toki-shakuyaku-san, Trikatu, Unkei-to, Yukmijihwang-tang
Analgesic 343.3 ± 226.6 (33)	Single: *Aconitum carmichaelii* Debeaux, *Bryophyllum pinnatum* (Lam.) Oken, *Dioscorea villosa* L., *Momordica cochinchinensis* (Lour.) Spreng., *Morinda citrifolia* L., *Panax ginseng* C.A.Mey., *Tephrosia purpurea* (L.) Pers. Multiple: Huo Luo Xiao Ling Dan, Joloo, NPI-028, Sokei-kakketsu-to, Toki-shakuyaku-san
Endocrine, nutritional and metabolic diseases 228.2 ± 123.6 (32)	Single: *Ajuga iva* (L.) Schreb., *Camellia sinensis* (L.) Kuntze, *Cassytha filiformis* L., *Piper sarmentosum* Roxb., *Rhododendron arboreum* Sm., *Smallanthus sonchifolius* (Poepp.) H.Rob. Multiple: ADD-199, Bangpungtongseong-san, Byakko-ka-ninjin-to, Choto-san, Dianex, Gosha jinki-gan, Hachimi-jio-gan, Keishi-bukuryo-gan, Toki-shakuyaku-san, Yukmijihwang-tang
Mental and behavioral disorders 391.6 ± 570.8 (25)	Single: *Ginkgo biloba* L., *Nelumbo nucifera* Gaertn., *Piper methysticum* G.Forst., *Stachys lavandulifolia* Vahl Multiple: Kai-Xin-San, PM012, Saiko-ka-ryukotsu-borei to, Yoku-kan-san-ka chimpi hange
Respiratory system 238.7 ± 222.5 (23)	Single: *Aristolochia contorta* Bunge, *Asarum heterotropoides* F.Schmidt, *Ephedra sinica* Stapf, *Piper sarmentosum* Roxb. Multiple: Gumiganghwal-tang, KOB03, Mahwangyounpae-tang, Ojeok-san, Saiboku- to, Saiko-keishi-to, Shigyaku-san, Trikatu
Infectious diseases 110.6 ± 118.6 (21)	Single: *Annickia chlorantha* (Oliv.) Setten & Maas, *Aristolochia contorta* Bunge, *Bryophyllum pinnatum* (Lam.) Oken, *Carica papaya* L., *Echinophora platyloba* DC., *Nelumbo nucifera* Gaertn., *Ocimum gratissimum* L., *Piper sarmentosum* Roxb., *Pteris multifida* Poir., *Stachys lavandulifolia* Vahl Multiple: Muhanse M4
Neoplasms 163.1 ± 116.4 (21)	Single: *Aster squamatus* (Spreng.) Hieron., *Bryophyllum pinnatum* (Lam.) Oken, *Morinda citrifolia* L., *Tephrosia purpurea* (L.) Pers. Multiple: Amukkarac curanam, Bojungbangdock-tang, Dangguibohyel-tang, Joloo, Sagunja-tang, Sipjeondaebo-tang, Soshiho-tang
Revitalization 433.0 ± 265.2 (20)	Single: *Panax ginseng* C.A.Mey., *Polygonum minus* Huds. Multiple: Dangguibohyel-tang, Homnawakod, Myelophil, Palmul-tang, Samul-tang
Circulatory system 205.5 ± 187.0 (19)	Single: *Aconitum carmichaelii* Debeaux, *Momordica cochinchinensis* (Lour.) Spreng., *Petroselinum crispum* (Mill.) Fuss Multiple: BDR-29, Choto-san, HMC05, Modified Wenpitang-Hab-Wulingsan, Oren-gedoku-to, Prototype-cheonggukjang, Sagunja-tang, WK-38
Others 738.8 ± 705.8 (11)	Single: *Paullinia pinnata* L., *Siraitia grosvenori* (Swingle) C. Jeffrey ex A.M. Lu and Zhi Y. Zhang. Multiple: Aristolochiae fructus mixture, GutMotil, PartySmart

[a] Some drugs were classified in multiple indication categories. Analysis of variance showed significant differences in HED values among the 11 drug indications.
[b] The mean ± standard deviation is expressed as mg/kg/day.

(40.3 mg/kg of HED) having an approximately seven to eight-fold of average HED value (278.1 mg/kg) had been reported to induce toxicity in human (Kim et al., 2013; Bujanda et al., 2002).

HED values differed according to drug classification by clinical use: drugs for revitalization showed the highest HED value (433.0 ± 265.2 mg/kg), and those for infectious diseases the lowest (110.6 ± 118.6 mg/kg). Drugs for revitalization such as *Panax ginseng* C.A. Meyer traditionally have a wide spectrum of users, including healthy subjects, and are believed to be nontoxic (Cho, 2000; Lee and Son, 2011). Herbal drugs for infectious diseases had the lowest mean HED value (110.6 ± 118.6 mg/kg), including *Annickia chlorantha* (Oliv.) Setten & Maas (1.6 mg/kg HED value, Moody et al., 2007) and *Echinophora platyloba* DC (8.1 mg/kg HED value, Mirghazanfari et al., 2012). As shown in our data, however, standard deviations are too large to draw a conclusive HED value. In fact, it is difficult to assign simply an average HED to a group of herbal medicines because each of these herbal medicines has the particular therapeutic target. Therefore any trend toward lower HED for certain herbs should be carefully interpreted.

As expected, no significant difference in HED value was observed between males and females. We also searched 244 randomized controlled trials (RCTs) of herbal drugs, revealing a mean HED of 70.3 ± 160.8 mg/kg (data not shown). Based on the latter finding, the average HED value of 278.1 ± 358.0 mg/kg in this survey study was about fourfold higher than the average clinical dose of herbal drugs. HED values are affected by the experimental design used to determine the highest dose; thus, only 24.9% of the 233 studies in our survey had lower NOAEL values than the highest experimental dose, indicating that the real HED values would be higher than those reported herein.

The present study possessed some limitations. This review included data in journals which are indexed internationally and Korean database, and included an insufficient number of toxicity studies compared with the numerous herbal drugs and medicinal

plants available, as well as limited clinical interpretation of the NOAEL and HED values. Nevertheless, this study is the first to report the HED values of herbal drugs.

In summary, the total average HED value of herbal drugs was 278.1 ± 358.0 mg/kg which was highest in herbal drugs for revitalization use and lowest in herbs for infectious diseases. These data will be a helpful reference for the development and clinical application of drugs.

Declaration of conflicting interests

The authors declare no conflict of interest.

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Appendix A. Supporting information

Supplementary data associated with this article can be found in the online version at http://dx.doi.org/10.1016/j.jep.2014.12.023.

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